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PATENT

Attorney Reference Number 4239-66899-01
Application Number 10/666,022

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Klinman et al.
Application No. 10/666,022
Filed: September 17, 2003
Confirmation No. 7954
For: METHOD OF TREATING AND PREVENTING INFECTIONS IN IMMUNOCOMPROMISED SUBJECTS WITH IMMUNOSTIMULATORY CPG OLIGONUCLEOTIDES
Examiner: Nita Minnifield
Art Unit: 1645
Attorney Reference No. 4239-66899-01

SUBMITTED VIA EFS ON
June 5, 2008

COMMISSIONER FOR PATENTS
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DECLARATION OF DANIELA VERTHELYI UNDER 37 C.F.R. § 1.132

1. I, Daniela Verthelyi, am an inventor of the above-identified patent application. I currently hold the position of Supervisory Biologist of the Laboratory of Immunology at the National Institutes of Health. I hold a Ph.D. from the Virginia Polytechnic Institute and State University and an M.D. from University of Buenos Ares and had my post-doctoral training in the area of Microbiology and Immunology. I am an expert in the fields of Biology and Immunology. A copy of my curriculum vitae is attached.

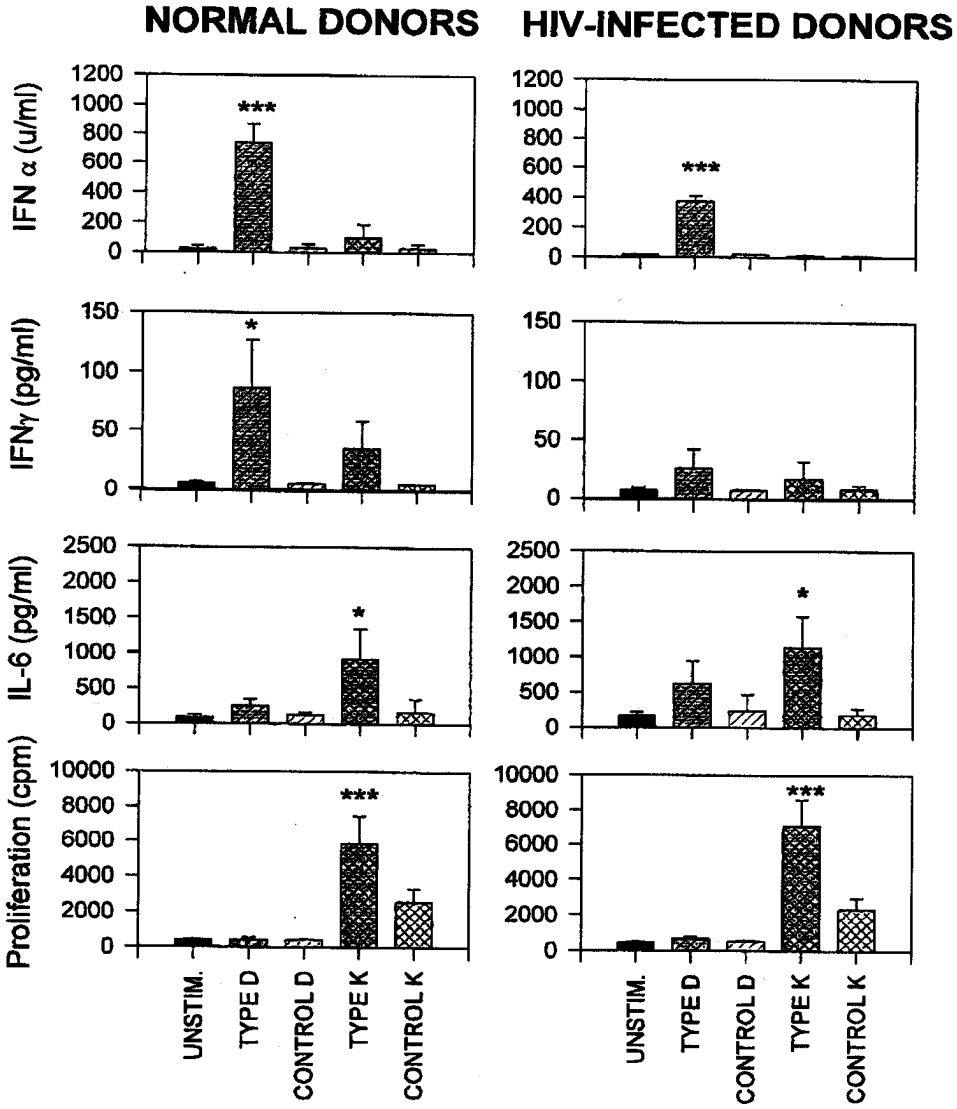
2. I have read the specification of the above-referenced application, and the Office action, dated December 6, 2007. It is my understanding that claims 1-21 and 25-28 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly not being enabled by the specification. The Office action alleges that a single D oliogdeoxynucleotide (ODN) would not be effective at producing an immune response in an immunocompromised subject. The Office action alleges that a successful cocktail of ODNs could not be designed based on the guidance in

the specification. The following data document the effectiveness of mixtures of ODNs and a single ODN to induce an immune response to an opportunistic infection in an immunocompromised host, such as the production of an immune response to an infection, such as Leishmainia, in a subject with a compromised immune system such as infected with a human or simian immunodeficiency virus (HIV or SIV, respectively). The sequences of all of the ODNs discussed below are provided in the specification of the present application, such as SEQ ID NOs: 176-181; see also page 45.

3. Previous studies from our lab have shown that individual humans and monkeys vary in their response to specific "K" and "D" sequences. No single "D" or "K" motif is optimally stimulatory in all donors. However, mixtures of ODN were identified that strongly stimulated peripheral blood mononuclear cells (PBMC) from all human donors (Leifer *et al.*, Heterogeneity in the human response to immunostimulatory CpG oligodeoxynucleotides, *J Immunother.* 26(4):313-9, 2003). These D or K ODN mixtures were used to produce an immune response to an opportunistic infection in immunocompromised macaques *in vivo*.

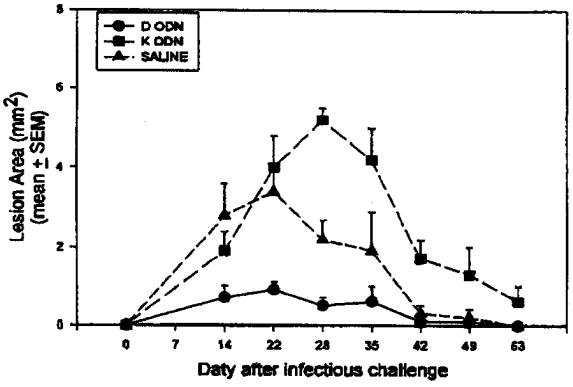
ODN with the following sequences were used: D19: GGtgcatcgatgcagGGGGG, D35: GGtgcatcgatgcaggggGG and D29 : GGtgcaccggtgcagGGGGG K3: ATCGACTCTCGAGCGTTCTC, K123: TCGTTTGTTCT and K23: TCGAGCGTTCTC (phosphorothioate bases in capital letters, phosphodiester bases in small case; underlining shows CpG motif). Control ODN included: D122 GGtgcattgatgcagGGGGG and K163: TGCAGGCTTCTC. These ODNs are all disclosed in the specification on page 45.

PBMC from HIV infected and healthy subjects responded similarly to K type ODN (Figure 1), suggesting that B cells and monocytes retained their ability to respond to this form of immune stimulation. Although D type ODN induced a significant increase in cytokine secretion by cells from both donor populations ($p < 0.001$), the IFNγ response of healthy controls significantly exceeded that of HIV-infected subjects ($p<0.05$ and $p<0.001$, respectively, Figure 1). This reduced responsiveness to D ODN correlated directly with the number of $CD4^+$T cells among the HIV infected donors ($p<0.01$) and inversely with their viral load ($p<0.05$).



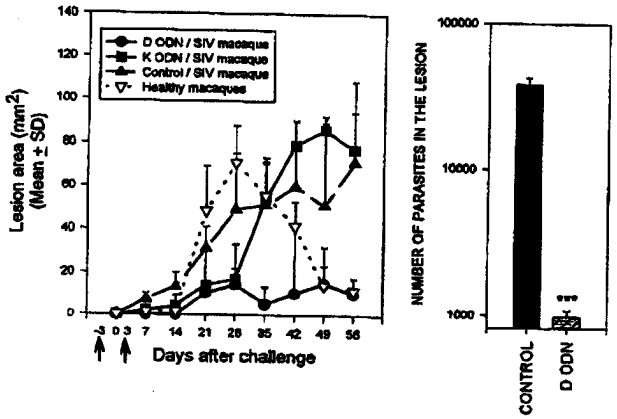
D ODN also maintained their ability to trigger the maturation of DC from HIV-infected donors. The absolute number of mature DC was lower in the unstimulated PBMC from HIV infected than in normal donors (0.13± 0.06% vs 0.26± 0.07 respectively, $p<0.05$). However, treatment with D ODN increased the number of CD83+, CD86+ cells by approximately 20-fold (to 2 +1 % vs 6 + 1 %) in both groups.

3. Rhesus macaques are known to provide a model for evaluating compositions for human use. A self-limiting cutaneous *Leishmania amazonensis* challenge model was used to evaluate whether CpG ODN could protect rhesus macaques from an opportunistic infection with Leishmania. Macaques were injected intradermally (i.d.) on days -3 and 3 with 500 ug of CpG ODN. On day 0, the animals were challenged at the same site (forehead) with 10^7 metacyclic *L. amazonensis* promastigotes. Naive animals developed a cutaneous lesion similar to those found in human cutaneous leishmaniasis with a peak surface area of 4.4 ± 0.7 mm^2 on day 22 (Figure 2). Lesion size was significantly reduced among macaques treated with D type ODN ($p<0.001$, Figure 2).

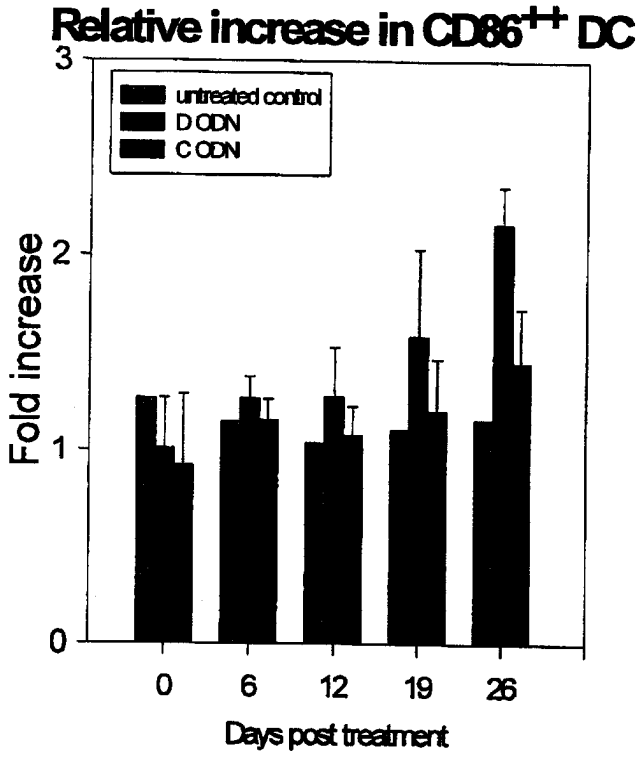


4. In another experiment, macaques that had been infected >12 months earlier with SIV Mac239 and had viral loads ranging from 0.3-28 $x10^6$ copies/ml were used. The animals were stratified based on viral load and then challenged with *L. major* metacyclic promastigotes (MHOM/IL/80/Friedlin). As shown previously, healthy macaques challenged with L. major develop cutaneous lesions characterized by erythema, induration and ulceration that peaked 25 days after challenge and resolved within 50 days (Fig. 3A). Due to their immunosuppressed state, macaques not treated with D ODN developed severe progressive cutaneous that did not resolve. The severity of *Leishmania* infection in SIV-infected animals treated with K ODN was not significantly different from that of the controls. In contrast, SIV-infected macaques treated

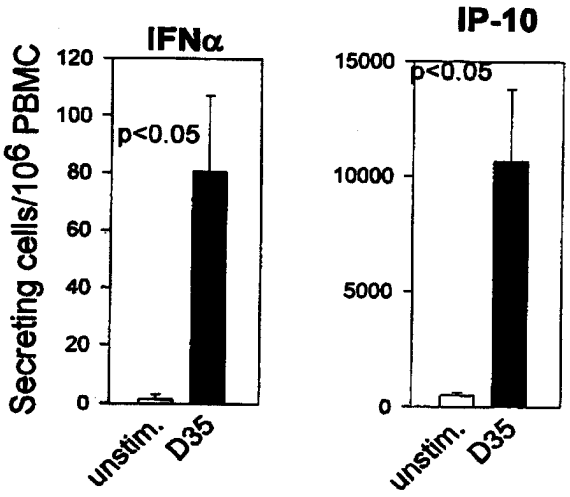
with D ODN developed significantly smaller lesions, and their infection did not progress over time (Figure 3A) as compared to controls. The animals were euthanized on day 56, and their parasite burden measured. SIV-infected monkeys treated with D ODN had a 35-fold reduction in total parasite burden at the lesion site compared to SIV infected animals treated with control ODN or saline (Fig. 3B, $p<0.001$). No systemic spread of the parasites were evident on any of the groups.



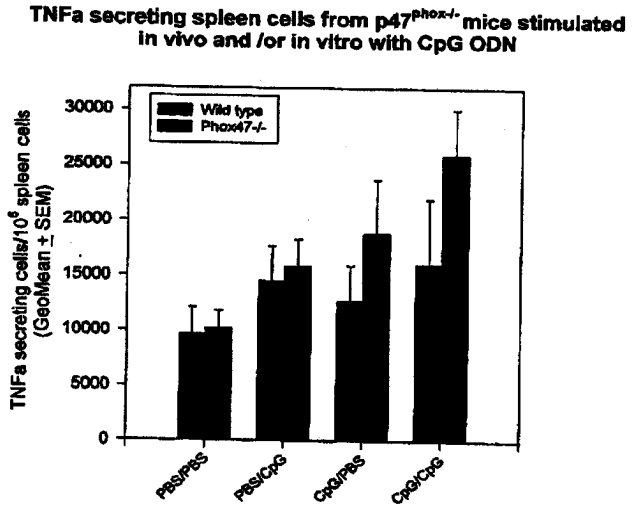
5. Although mixtures of D ODN can be used to induce an immune response to an opportunistic infection in an immunocompromised host, an individual ODN could be utilized as well. Different D ODNs motifs can produce an increased or decreased effect (quantitative difference) in different donors. Data was obtained documenting that individual D ODNs could be used to produce an immune response in immunocompromised subjects. SIV-infected macaques were treated with 0.5mg/kg of D35 (SEQ ID NO: 177) three times a day for three days (n=3/group). Although there was no change in the number of CD4 or CD8 cells, there was an increase in $CD14^{-}$ $CD19^{-}CD86^{bright}$ dendritic cells, indicating an increase in activated DC in peripheral blood (see FIG. 4).



In addition, IFN-γ and IP-10 secretion was increased in healthy macaques treated with a single D ODN (D35, SEQ ID NO: 177, also encompassed in the general formula set forth in SEQ ID NO: 22), see Fig. 5 below.

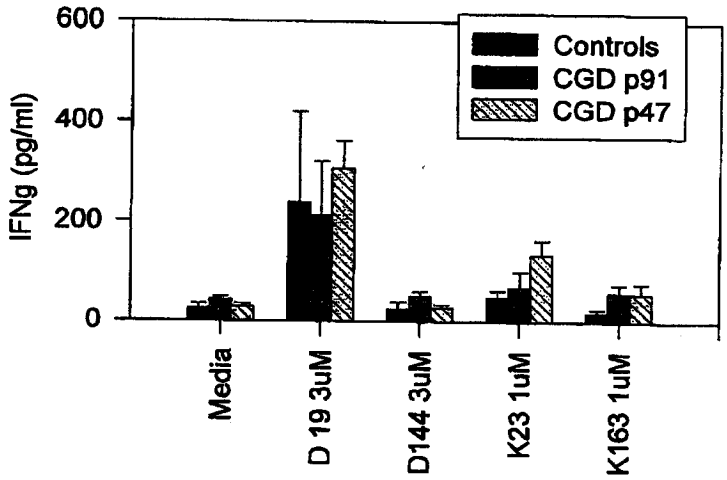


6. A single CpG ODN (ODN1555, SEQ ID NO: 22) was administered to p47phox-/- mice, which exhibit a phenotype similar to that of human chronic granulomatous disease (CGD). The biochemical basis for CGD is a defect in the phagocyte nicotine amide dinucleotide phosphatase (NADPH) oxidase, the enzyme responsible for producing superoxide O^{-2}, which in turn is critical for host defense against bacterial and fungal infection. Mice were treated with CpG (ODN 1555). Mice treated with CpG D ODNs showed an increased production of tumor necrosis factor alpha (TNFa) and interleukin12 (IL-12) as compared to those mice treated with phosphate buffered saline, indicating that this D ODN can be used to include an immune response, such as to an opportunistic infection. The results for TNFa production are shown in Fig. 6 below.



7. Human patients with CGD have NADPH oxidase defect which results in the defective generation of reactive oxygen species. This disease is associated with infections by S. aureus, Salmonella, Pseudomonas, Burkhoderia, Serratia, Candida, or Aspergillus leading to septicemia. PBMC from these patients were treated with D ODN individually. Treatment of PBMC from subjects with chronic granulomatous disease (CGD) with either D19 (SEQ ID NO: 176, see also SEQ ID NO: 22) induced production of IFN-γ and IL-16-at a level similar to healthy controls. The results for IFN-γ are shown below in Fig. 8. This indicates the D ODN can be used to

activate an immune response in a subject with CGD, such as to improve the immune response to an opportunistic infection.



8. The results presented above provided confirmatory evidence that mixtures of D ODN and individual D ODN, can be used to produce an immune response in art-recognized models of immunocompromised subjects, including models of HIV infection and CGD. All of the results were obtained using ODNs and model systems fully described in the above-referenced patent application. These results document that one of skill in the art could readily make and use the claimed methods using the guidance provided in the specification.

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of the Title 18 of the United

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States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Daniela Verthelyi, M.D., Ph.D.

6-3-08
Date